HILGARDIA

A JOURNAL OF AGRICULTURAL SCIENCE

PUBLISHED BY THE

CALIFORNIA AGRICULTURAL EXPERIMENT STATION

VOL. 1 DECEMBER, 1925 No. 13

THE RÔLE OF ACIDITY IN VEGETABLE CANNING

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Present industrial methods of sterilizing canned vegetables of low acidity result in considerable injury to texture, flavor, and color because of the high temperatures and long periods of heating necessary to destroy heat resistant organisms. Because of the lack of proper facilities, it is not feasible in many homes, to apply the temperatures necessary to sterilize vegetables, and heating for one to three hours, at the temperature of boiling water, a method formerly recommended for home use, has been proved unsafe because it does not always destroy the spores of *B. botulinus.*

It is a well recognized fact that vegetables of high acidity, such as rhubarb and tomatoes, are easily sterilized. It is also well known that the addition of dilute organic acids to the brines used in canning makes it possible to preserve vegetables of low acidity by heating at 100° C.

However, previous investigations showed considerable variation in the effect of added acid on the sterilization of various vegetables. Not all vegetables behaved alike. Preliminary observations also showed that at least some of the variations observed could be traced to marked changes in pH value of the acidified brines during heating. Therefore, one part of the present investigation was to determine the magnitude of these changes in pH value.

A second part was to determine more accurately than had been done previously the effect of acidified brines on the sterilization of vegetables of low acidity artificially contaminated with large numbers of heat resistant microörganisms.

REVIEW OF THE LITERATURE

Experiments conducted by Cruess in 1913–1915 and reported briefly in Circular 158[1] of this station in 1916 showed that peas, string beans, pumpkins, beets, turnips, artichokes, and asparagus canned in brine acidified with 4 to 6 ounces of lemon juice per gallon and processed one hour at 212° F., kept perfectly for an incubation period of more than a year, while the same vegetables canned in the same manner but in non-acidified brines spoiled. The samples were not artificially inoculated with heat resistant organisms, but the fact that the non-acidified checks spoiled showed that such organisms were present naturally.

Dickson,[2] Burke and Ward conducted experiments in 1917 and 1918 on the effect of acidified brine on the growth of *B. botulinus* and on the destruction of its spores by heat in canned vegetables. Their conclusions are summarized in the following quotation:

> Our first series of experiments show beyond all doubt that when exposed to brines containing lemon juice in the amount of approximately 5 per cent, a virulent toxin is developed under favoring conditions of temperature. But when the spores are exposed to the action of boiling water for one hour in acid brines of similar concentrations, as in the method of canning recommended by Cruess or in a mixture of lemon juice of more than 2 per cent, as in our preliminary experiments, the spores are completely destroyed.

Their control samples, not acidified, but heated for one hour in boiling water developed growth of *B. botulinus* and a strong toxin.

Skinner and Glasgow[3] report that the addition of two tablespoons of vinegar of 4.4 per cent total acid (as acetic) per quart of brine used in canning asparagus greatly reduced the time necessary for sterilization at 100° C.

Weiss[4] determined the effect of pH value on the death point of the spores of a resistant strain of *B. botulinus*. In citric acid of a pH value of 3.16 (2.1 per cent acid) the spores were killed in less than 10 minutes at 100° C., while the time required at pH 6.66 (nearly neutral) was 90 minutes.

The same investigator[5] found that the spores of *B. botulinus* were killed at 100° C. in eleven different food juices having a pH range of 2.1 to 3.81 in less than 50 minutes, in 60 to 90 minutes in those of pH 4.22 to 4.4, in 90 to 120 minutes in those of pH 5.13 to 5.36, while those of pH 5.69 to 6.21 required 150 to 180 minutes.

Using spores of thermophiles that are extremely resistant to heat Bigelow[6] and Esty determined the death times in juices expressed from various commercially canned vegetables at 100°, 110°, 115°, and 120° C. The time necessary to kill the spores of the most resistant organism at 100° C. was as follows: 1200 minutes in corn juice of pH 6.1; 1020 minutes in pea juice of pH 5.3; 360 minutes in sweet potato juice of pH 5.0; 210 minutes in spinach juice of pH 5.0; 360 minutes in string bean juice of pH 5.0; 210 minutes in beet juice of pH 4.7, and 210 minutes in pumpkin juice of pH 4.5.

Dickson[7] and his associates in 1922 reported experiments in which the heat resistances of *B. botulinus* spores in various concentrations of hydrochloric, citric, acetic, and lactic acids were determined. At corresponding pH values, there seemed to be little difference in the toxic effect of the various acids. The OH ion also exerted a toxic effect, as it was found that the heat resistance was much less in alkaline than in neutral solutions.

Esty[8] and Meyer conducted an extensive series of experiments to determine the effect of hydrogen ion concentration on the death point of *B. botulinus* in phosphate solutions, Difco pepton solutions, double strength veal infusion, spinach juice, and in juices expressed from many canned foods. Because of the action of buffer substances, much more acid was required to give the desired pH values in spinach juice than in phosphate and peptone solutions.

The spores in a citric acid solution of pH 5.26 were killed in 65 minutes at 100° C., at pH 4.69 in 40 minutes; at pH 4.31 in 20 to 25 minutes, while at pH 7 about 330 minutes was required.

Bigelow[9] and Cathcart in studying the changes in hydrogen ion concentration occurring during the processing of many canned foods found that the pH value decreased slightly in most cases on account, they believed, of the coagulation of buffer substances and the formation of weak acids through decomposition of organic compounds by heat. In one experiment, however, that with beans in tomato sauce, there was an increase in pH value in the sauce which the authors state was "due to diffusion of acid into the beans." Undoubtedly, part of the change was due to this cause; but we believe, from our experiments with other products, that some of it may have been due to the buffer action of compounds leached from the beans during processing.

CHANGES IN HYDROGEN ION CONCENTRATION DURING CANNING

The foregoing review of the literature shows that a number of investigators in several laboratories have studied the effect of pH value on the death point of heat resistant organisms, but that little has been published on the changes occurring in the pH value of acidified brines in canned vegetables during actual canning and sterilizing operations. Unless the extent of these changes is known, it is impossible to specify what the hydrogen ion concentration of the acidified brine should be at the time of its addition to the product. Therefore, in our investigations, we gave as much attention to this phase of the problem as to tests on the effect of pH value on the death point of heat resistant spores.

1. *Procedure.*—Various vegetables were prepared for canning in the usual manner and as described later. All of the brines used in the experiments, except those for corn, consisted of 2 per cent of salt in distilled water plus various concentrations of hydrochloric, citric, or acetic acids. The brine for corn contained 2 per cent of salt and 5 per cent of sugar in addition to the added acid.

The pH values of the solutions when prepared for use in the canning tests were carefully checked colorimetrically by the Clark and Lubs[10] method against standardized buffer solutions of known pH values.

In the first year's experiments number 2 cans were used, filled to the usual height with the same weight of vegetables. They were then filled with the brine, but this was not measured. In later experiments 8-ounce cans were used. Weighed amounts of vegetables and measured amounts of brine were added. Eighty grams of sweet corn plus 125 c. c. of brine; 120 grams of string beans plus 100 c. c. of brine; 180 grams of spinach plus 25 c. c. of brine; and 130 grams of asparagus plus 90 c. c. of brine were the ratios used. The later experiments, therefore, can be more easily duplicated.

The filled cans were heated in live steam at 99° to 110° C. for 5 to 8 minutes before sealing, the time varying with the size of container and character of the product.

''Sanitary'' (open top) cans were used and were sealed with a hand roll, foot pressure, power driven double-seamer. Tests showed that the sealing operations were satisfactory.

''Processing'' in all investigations reported in this publication was conducted with boiling water at approximately 100° C. Various time periods varying from one-half to two hours were used.

After exhausting and again after processing for the various times, samples of the brines from the cans were taken, filtered, and their pH values determined colorimetrically.

2. *Relative Changes in pH Value during Exhausting and Processing.*—Changes in pH value of acidified brines in cans of vegetables were greater during exhausting before sealing than during processing in the sealed cans. All acidified brines increased in pH value during exhausting and most brines increased during processing.

Table 1 illustrates the relative changes that were observed in several experiments during exhausting and processing. The table presents only a small proportion of the data obtained.

TABLE 1

TYPICAL pH VALUE CHANGES DURING EXHAUSTING AND PROCESSING OF BRINES ACIDIFIED WITH CITRIC ACID

Vegetable and pH value of its juice	pH value of original brine	pH value of brine after exhausting 5–8 minutes	pH value of brine after 60 minutes processing at 100° C.
Sweet corn, pH 6.8	2.8	3.6	4.2
Sweet corn, pH 6.8	4.0	5.6	5.2
String beans, pH 6.2	2.8	3.6	3.8
String beans, pH 6.2	4.0	5.8	5.4
String beans, pH 6.2	6.0	6.0	5.4
String beans, pH 6.2	7.0	6.0	5.3
Spinach, pH 6.8	2.8	3.0	4.0
Asparagus, pH 5.4	2.8	3.9	4.0
Asparagus, pH 5.4	3.6	4.6	4.5

Possibly in those acidified brines in which the pH value increased during exhausting but later decreased during processing, buffer substances were precipitated or decomposed, or acids or acid salts were formed. Continued increase of pH value during processing was probably due to diffusion because it is probable that during exhausting most of the buffer effect occurred from compounds dissolved from the vegetables.

3. *Effect of Length of Processing on pH Value.*—Several vegetables were processed for 20, 40, 60, and 80 minutes in order to determine the effect of the length of processing at 100° C. on pH changes in acidified brines. Some of the data are presented in table 2.

After exhausting and the first 20 minutes of processing, changes in pH value were slight in most instances.

TABLE 2

EFFECT OF LENGTH OF PROCESSING ON CHANGES IN pH VALUE OF BRINES ACIDIFIED WITD CITRIC ACID

Vegetable and pH value of its juice	pH value of original brine	pH value after processing at 100° C.			
		20 minutes	40 minutes	60 minutes	80 minutes
Sweet corn, pH 6.8	2.0	2.9	3.0	3.2	3.2
Sweet corn, pH 6.8	2.8	4.0	4.2	4.2	4.2
Sweet corn, pH 6.8	4.0	5.4	5.2	5.2	5.2
Sweet corn, pH 6.8 (Not acidified)	7.0	6.5	6.2	6.2	6.1
String beans, pH 6.2	2.0	3.2	3.2	3.2	3.0
String beans, pH 6.2	4.0	5.4	5.4	5.4	5.4
Asparagus, pH 5.4 (1924)	2.0	3.6	3.5	3.2	3.1
Asparagus, pH 5.4 "	3.6	4.4	4.4	4.5	4.6

TABLE 3

CHANGES IN pH VALUE OF ACIDIFIED BRINES DURING PROCESSING AS AFFECTED BY TYPE OF ACID

Vegetable	Acid used	Original pH value of brine	pH value of brine after 1 hour at 100° C.
Asparagus (1923)	None	7.0	5.6
Asparagus "	Hydrochloric	2.6	5.4
Asparagus "	Citric	2.6	5.1
Asparagus "	Acetic	2.6	4.6
Asparagus "	Hydrochloric	3.6	5.6
Asparagus "	Citric	3.6	5.6
Asparagus "	Acetic	3.6	5.4
Sweet corn	None	7.0	6.2
Sweet corn	Hydrochloric	2.0	5.0
Sweet corn	Citric	2.0	3.2
Sweet corn	Hydrochloric	4.0	5.6
Sweet corn	Citric	4.0	5.2
String beans	None	7.0	5.4
String beans	Hydrochloric	2.0	4.8
String beans	Citric	2.0	3.2
String beans	Hydrochloric	2.8	4.4
String beans	Citric	2.8	3.8
String beans	Hydrochloric	4.0	5.4
String beans	Citric	4.0	5.4
Peas, green	Hydrochloric	2.6	5.8
Peas, green	Citric	2.6	5.4
Peas, green	Acetic	2.6	4.8
Peas, green	Hydrochloric	3.0	6.0
Peas, green	Citric	3.0	5.7
Peas, green	Acetic	3.0	5.4

4. *Comparison of Different Acids.*—A considerable number of experiments were made to determine the relative changes in pH value occurring in brines acidified with hydrochloric, citric, and acetic acids, respectively. In table 3 some of the data obtained are presented.

In brines of relatively low original pH value, acetic acid gave pH values after processing that were lower than those acidified with citric acid, and citric acid in turn lower than hydrochloric acid. These observed differences are very probably due to the differences in degrees of ionization of the different acids.

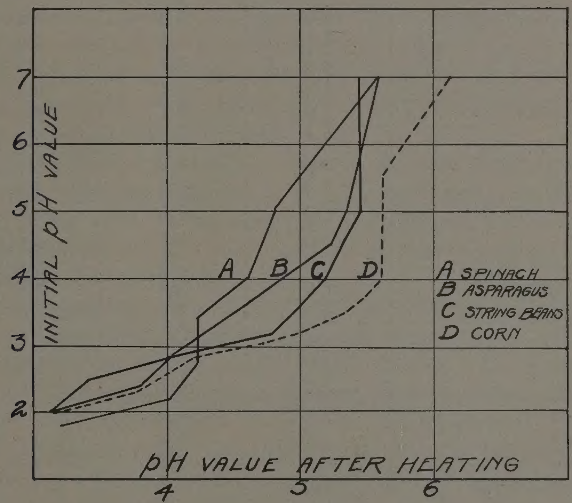

Fig. 1. Changes in pH value of acidified brines during processing at 100° C.

For practical purposes, citric is to be preferred to acetic acid because it does not affect the flavor so noticeably.

Three per cent of lemon juice added to brine gave a pH value of 2.9 before processing and a pH on carrots after processing of 5.0. For a 5 per cent addition of lemon juice, the figures were 2.7 and 4.8, respectively. A brine acidified to pH 3.0 with citric acid increased to 5.2 during processing on carrots. Apparently the change during processing is about equal with brines of equal original pH value whether citric acid or lemon juice is used.

5. *Comparison of Different Vegetables.*—Typical changes in pH value in brines on asparagus, corn, string beans, and spinach are shown in tables 3 and 4 and figure 1. There was some variation in pH

changes in different lots of the same vegetable, probably occasioned by differences in their chemical composition; e.g., see asparagus in tables 4 and 5. Usually this variation did not exceed .2 pH.

TABLE 4

RELATIVE EFFECT OF DIFFERENT VEGETABLES ON CHANGES IN pH VALUE OF BRINES ACIDIFIED WITH CITRIC ACID. (See also table 3.)

Vegetable	pH value of original brine	pH value of brine after processing 1 hour at 100° C.	Ratio of vegetable to brine
Carrots	3.0	5.2	2 : 1 approx.
Carrots	3.8	5.4	2 : 1 approx.
Carrots	4.4	5.4	2 : 1 approx.
Carrots	4.8	5.4	2 : 1 approx.
Carrots (check)	7.0	5.4	2 : 1 approx.
Peas (green)	3.0	5.7	2 : 1 approx.
Peas (green)	3.6	6.0	2 : 1 approx.
Peas (check)	7.0	6.0	2 : 1 approx.
Artichokes	2.6	4.6	1 : 1 approx.
Artichokes	3.0	4.8	1 : 1 approx.
Artichokes	3.6	5.3	1 : 1 approx.
Artichokes (check)	7.0	5.3	1 : 1 approx.
Sweet corn	2.0	3.2	0.64 : 1
Sweet corn	2.8	4.2	0.64 : 1
Sweet corn	4.0	5.6	0.64 : 1
Sweet corn	7.0	6.2	0.64 : 1
String beans	2.0	3.2	1.2 : 1
String beans	4.0	5.2	1.2 : 1
String beans	6.0	5.4	1.2 : 1
String beans (check)	7.0	5.4	1.2 : 1
Spinach	2.8	4.0	7 : 1
Spinach	3.4	4.2	7 : 1
Spinach	3.8	4.4	7 : 1
Spinach	7.0	5.6	7 : 1
Asparagus	2.0	3.2	1.4 : 1
Asparagus	2.8	4.0	1.4 : 1
Asparagus	3.6	4.5	1.4 : 1
Asparagus	5.0	5.4	1.4 : 1
Asparagus	7.0	5.4	1.4 : 1

Evidently peas possess a higher concentration of buffer substances than do the other vegetables. It would appear that the other vegetables named in the table exert about an equal effect on the pH value of acidified brines, at least on brines of low to moderate pH value. At high pH values differences are more pronounced.

EFFECT OF pH VALUE ON THE DEATH POINT OF RESISTANT BACTERIAL SPORES

Experiments were conducted with three heat resistant organisms, *B. sporogenes, B. botulinus*, and a thermophile and with six varieties of vegetables, asparagus, artichokes, sweet corn, peas, spinach, and string beans in order to obtain information on the effect of pH value on the death points of these organisms under practical canning conditions.

1. *Procedure.*—In the preliminary experiments in 1917 and 1918 brain medium cultures of four strains of *B. botulinus* from Dr. I. C. Hall of the Bacteriology Department, University of California, were mixed and used for inoculation of canned peas, corn, and string beans. Each culture contained abundant spores, but their heat resistance by test tube tests was not determined.

In the 1923–24 experiments, 4-day old cultures of a heat resistant strain of *B. sporogenes* grown at 37° C. were used; and 3-day old cultures in the 1924–25 experiments. The cultures were diluted 1:3 with sterile water and 1 c. c. of the diluted culture used to inoculate each 8-ounce can of vegetable.

A resistant strain of a thermophile was obtained from Dr. J. R. Esty of the National Canners' Research Laboratory, Washington, D. C., through the courtesy of Dr. K. F. Meyer. It was grown at 55° C., on nutrient agar slants and the organisms were washed from the agar with sterile water to give a rich suspension of spores. One c. c. of the suspension was used for each 8-ounce can of vegetable.

A limited number of tests were made also with a suspension of resistant *B. botulinus* spores furnished by Dr. K. F. Meyer. This suspension in our experiments was diluted 1:5, and 1 c. c. of the diluted suspension was used for inoculation of each 8-ounce can.

In a special meat medium of approximately pH 7, the *B. sporogenes* spores used in the 1923–24 experiments survived 180 minutes at 100° C., but were killed in 195 minutes. In 2 per cent glucose broth of pH 7 they survived 165 minutes at 100° C., but were killed in 180 minutes.

The *B. sporogenes* spores used in the 1924–25 experiments were killed in 165 minutes in the meat medium and in 135 minutes in the glucose broth at 100° C.

The thermophile spores were killed in from 315 to 350 minutes in glucose broth of pH 7—the resistance varying somewhat with the cultures used. The *B. botulinus* spores survived 275 minutes but were killed in 300 minutes at 100° C., in glucose broth of pH 7.

As a further check on the heat resistance in non-acidified media, inoculated but non-acidified samples of the canned vegetables were prepared in each expériment. In order to enable us to interpret our results on heat resistance more intelligently the rates of heat penetration in 8-ounce cans of experimentally packed string beans, corn, asparagus, spinach, and commercially canned creamed corn at 100° C., were measured by means of thermocouples.* Figure 2 gives the results of these measurements.

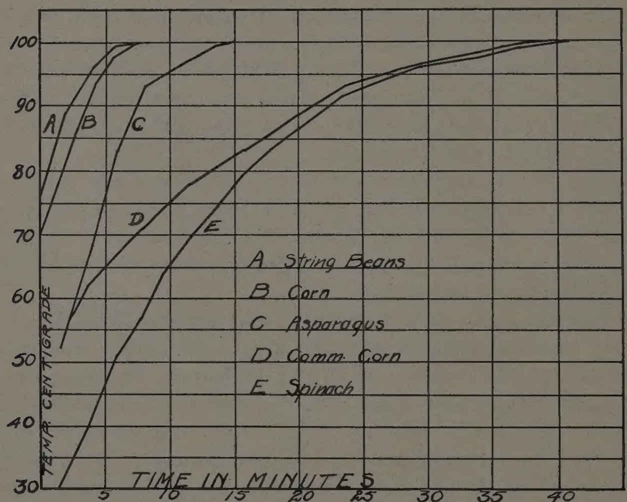

Fig. 2. Heat penetration in 8-ounce cans of string beans, corn, asparagus, commercially canned corn, and spinach.

Heat penetration in spinach was extremely slow, about 40 minutes at 100° C. being necessary for the center of the can to reach 99° C. The other vegetables tested reached the temperature of the bath very quickly.

2. *Effect of Acidified Brines on Heat Resistance of B. sporogenes.*—The data can be most conveniently considered according to the variety of vegetable used.

(*a*) *Asparagus:* Tests with this vegetable were made during both the 1923–24 and the 1924–25 seasons. The data are given in table 5. Three cans were used for each concentration in the 1923–24 experiments and four cans for each in the 1924–25 experiments.

* These tests were made in coöperation with J. Parcell.

The asparagus was cut to fit the cans, washed and then blanched 5 minutes in boiling water. It was not graded for size. The spores were added before the can was sealed and before exhautsing. The cans were then filled with brines of the desired pH values, exhausted 5 minutes in live steam and sealed immediately. They were put at once into boiling water and heated for the length of time shown in table 5. Number 2 cans were used in the 1923-24 season and 8-ounce cans in the 1924–25 season.

The cans were chilled completely to room temperature in cold water after removal from the processing vessel and then incubated at 37° C. for 3 months in the 1923–24 experiments and about 2½ months in the 1924–25 experiments. Spoiling was evidenced by swelling of the cans, but was confirmed by examination of the contents of the cans and by making transfers to nutrient media.

The data of the two seasons agree in indicating that for brines of equivalent pH value, acetic acid is somewhat more effective than citric in lowering the heat resistance of *B. sporogenes.* The ratio of volume of brine to weight of asparagus was greater in the 1924–25 tests, which explains the smaller changes in pH values and lower indicated heat resistance in the 1924–25 tests. The 1923–24 ratio, however, more nearly represents commercial practice.

In a can tightly packed with asparagus, apparently an original pH of 2.6 or less is necessary to insure destruction of the *B. sporogenes* spores used in 2 hours at 100° C.

When the pH values after processing are compared there is found a closer correlation between the results of the two seasons' experiments. Apparently the critical pH value if determined after processing is about pH 4.6, as the organism failed to grow after 1 hour's processing at 100° C. in both seasons' tests, when the final pH value was 4.6 or less.

(*b*) *Artichokes:* Artichokes inoculated with *B. sporogenes,* canned in plain brine of pH 7 and processed 1 hour at 100° C. spoiled. Those canned in citric acid brines of pH 2.6, 3.0, and 3.6 and processed 1 hour at 100° C. did not spoil. The final pH values were 4.6, 4.8, and 5.3, respectively.

(*c*) *String Beans:* Growth of *B. sporogenes* failed to occur in string beans after 1 hour's heating at 100° C., in the 1923–24 experiments, even in non-acidified brine, although the pH value of the non-acidified brine after processing was 5.4. In the 1924–25 experiments, therefore, the processing periods were shortened to ½ and 1 hour respectively.

TABLE 5

EFFECT OF pH VALUE OF BRINE ON CANNED ASPARAGUS ON HEAT RESISTANCE OF *B. sporogenes*

Acid	pH value of original brine	pH value of brine after processing	Period of processing at 100° C. in hours	Per cent of cans spoiled
1923–24 SEASON				
Hydrochloric	2.6	5.4	1	0
Hydrochloric	2.6	5.4	2	33
Citric	2.6	5.1	1	33
Citric	2.6	5.1	2	0
Acetic	2.6	4.6	1	0
Acetic	2.6	4.6	2	0
Hydrochloric	3.0	5.5	1	100
Hydrochloric	3.0	5.5	2	33
Citric	3.0	5.4	1	66
Citric	3.0	5.4	2	0
Acetic	3.0	5.2	1	33
Acetic	3.0	5.2	2	0
Hydrochloric	3.6	5.6	1	100
Hydrochloric	3.6	5.6	2	100
Citric	3.6	5.6	1	100
Citric	3.6	5.6	2	66
Acetic	3.6	5.4	1	0
Acetic	3.6	5.4	2	0
None (check)	7.0	5.6	2	100
1924–25 SEASON				
Citric	2.0	3.1	½	0
Citric	2.0	3.1	1	0
Citric	2.4	3.4	½	0
Citric	2.4	3.4	1	0
Citric	2.8	4.0	½	0
Citric	2.8	4.0	1	0
Citric	3.6	4.6	½	25
Citric	3.6	4.6	1	0
Acetic	3.6	4.6	½	0
Acetic	3.6	4.6	1	0
Citric	4.5	5.2	½	50
Citric	4.5	5.2	1	25
Acetic	4.5	5.2	½	25
Acetic	4.5	5.2	1	25
Citric	5.0	5.3	½	50
Citric	5.0	5.3	1	50
Acetic	5.0	5.3	½	25
Acetic	5.0	5.3	1	0
None	7.0	5.4	½	100
None	7.0	5.4	1	75

Citric acid only was used for acidifying the brines. Spoiling did not occur even after boiling for only ½ an hour when the original pH was 3.2 or less and the final pH 4.8 or less. When brines of original pH 3.6, 4.0, and 4.5 were used, spoiling occurred after boiling for ½ an hour but not after 1 hour. At pH 5.0 or above spoiling occurred in all cases.

Evidently some factor other than pH value affects the heat resistance of *B. sporogenes* in string beans, because the spores were more quickly killed in string beans than in asparagus in brines which were of the same pH value after processing. Nevertheless the critical pH value—between 4.8 and 5.0—was not greatly different from that found for asparagus.

(*d*) *Sweet Corn:* Fresh sweet corn on the cob was purchased in the local wholesale market, husked, cut from the cob, canned in 8-ounce cans, inoculated as previously described and brines of various pH values were added. The brines in one series were acidified with hydrochloric and in another with citric acid. The amount of brine used was greater than in commercial practice in order to obtain rapid heat penetration (see figure 2).

Spoiling did not occur in cans to which citric acid brines of pH 3.2 or lower were added, whether boiled 1 or 2 hours. When brines of pH 3.6 or higher were used spoiling occurred in all cases after boiling for either 1 or 2 hours except at pH 3.6, heated for 2 hours. The pH values after processing were 5.0 and 5.4, respectively.

With hydrochloric acid brines, the results were similar. The critical pH value of the brine after processing appeared to be about pH 5.0 to 5.2, somewhat higher than with asparagus and string beans.

(*e*) *Peas* (1923–24 season): The peas were prepared for canning as in regular cannery practice by shelling and blanching in water. They were placed in number 2 cans and each can was inoculated with 10 c. c. of a suspension of a 4-day old spore culture of *B. sporogenes*—a heavier inoculation than was used for corn, string beans or spinach. Brines of three pH values and acidified with acetic, citric, and hydrochloric acids, respectively, were used (see table 6).

The critical pH value after processing was, for peas with a 1½ hour processing period at 100° C., about 5.4 with both citric and acetic acids. This corresponded to an original pH value of 3.0 for acetic acid and 2.6 for citric acid. The increase in pH value was greater with peas than with the other vegetables.

As in other experiments the critical pH value is affected by the length of processing.

(*f*) *Spinach:* Spinach from the local wholesale market was trimmed, washed, blanched five minutes and canned in 8-ounce cans, 180 grams per can. It was inoculated with 1 c. c. of a suspension of a 3-day old *B. sporogenes* spore culture and 25 c. c. of brine was added per can. In one series citric and in another acetic acid was used (see table 7).

TABLE 6

EFFECT OF ACIDIFIED BRINES ON HEAT RESISTANCE OF *B. sporogenes* IN CANNED PEAS. (1923–24 SEASON)

Acid	pH value of original brine	pH value of brine after processing	Period of processing in hours	Per cent of cans spoiled
Hydrochloric	2.6	5.8	1	100
Hydrochloric	2.6	5.8	1½	100
Citric	2.6	5.4	1	0
Citric	2.6	5.4	1½	0
Acetic	2.6	4.8	1	0
Acetic	2.6	4.8	1½	0
Hydrochloric	3.0	6.0	1	100
Hydrochloric	3.0	6.0	1½	100
Citric	3.0	5.7	1	100
Citric	3.0	5.7	1½	100
Acetic	3.0	5.4	1	33
Acetic	3.0	5.4	1½	0
Hydrochloric	3.6	6.0	1	100
Hydrochloric	3.6	6.0	1½	100
Citric	3.6	6.0	1	100
Citric	3.6	6.0	1½	100
Acetic	3.6	5.6	1	100
Acetic	3.6	5.6	1½	66
None (check)	7.0	6.0	1½	100

The results with acetic acid were virtually the same as with citric acid.

Heat penetration is slow in spinach, a condition that may account for some of the spoiling of samples of relatively low pH value. Thus spoiling occurred at pH 4.2 (referring to brine after processing). However, even allowing for heat penetration, decreased pH value of the brine was less effective in spinach than in the other vegetables studied. Probably the leaves matted together in the can and prevented penetration of the acid to all the spores.

Because of this latter possibility another experiment was made. Spinach was blanched in brine acidified with citric acid in one case and with acetic in another and was canned in brines of pH 2.0

to 6.0. The canned samples were inoculated heavily and processed 1 and 2 hours at 100° C. Spoiling did not occur in any sample. This experiment suggests a method of applying acidified brines in spinach canning commercially.

3. *Effect of Acidified Brines on Heat Resistance of Spores of a Thermophile.*—The spores of this organism withstood 315 minutes at 100° C. in glucose bouillon in a control test, but were killed in 350 minutes.

TABLE 7

EFFECT OF CITRIC ACID BRINES ON THE HEAT RESISTANCE OF *B. sporogenes* SPORES IN CANNED SPINACH

pH value of original brine	pH value of brine after processing	Period of processing at 100° C. in hours	Per cent of cans spoiled
1.8	3.2	1	0
1.8	3.2	2	0
2.2	4.0	1	0
2.2	4.0	2	0
2.8	4.2	1	25
2.8	4.2	2	0
3.4	4.2	1	100
3.4	4.2	2	75
4.0	4.6	1	100
4.0	4.6	2	100
5.0	4.8	1	100
5.0	4.8	2	100
7.0	5.6	1	100
7.0	5.6	2	100

Fresh corn was prepared and canned as describeed for the *B. sporogenes* experiments. Brines acidified with hydrochloric and with citric acids were added. The cans were processed at 100° C. for 1, 3, and 5 hours. Since the pH changes during processing have been given elsewhere the detailed data from this experiment will not be presented.

Where the pH value of the brine acidified with citric acid was greater than or equal to 5.4 after processing, spoiling occurred even after 5 hours boiling. At pH 5.2 and less, spoiling did not occur. With hydrochloric acid, spoiling occurred at pH 5.5 but not at 5.4, indicating that hydrochloric acid may be more toxic than citric.

One striking observation was that processing for 1 hour was as effective as 5 hours, when the critical pH value was reached.

4. *Effect of Acidified Brines on Heat Resistance of Spores of B. botulinus.*—The experiments with *B. botulinus* have not been so extensive as with *B. sporogenes.* However, enough was done to con-

firm in general the results reported by Meyer, Esty, Weiss, Dickson, and others (see review of literature).

Preliminary experiments were made in 1917 and 1918 in order to compare the "cold-pack-one-period" and the "lemon juice" methods of home canning. Four strains of *B. botulinus* growing in brain medium were mixed, shaken violently with sterile water, and centrifuged. Shaking with sterile water and centrifuging were repeated twice in order to remove some of the toxin and to break up the clumps of medium. Spores were numerous in all four cultures.

For some of the tests commercially canned vegetables were used; for others, the fresh vegetables. The canned and glass packed samples were inoculated heavily with the mixed spore suspension; heated at 100° C. for one hour, except check samples, and then incubated for 2½ months. Check samples were inoculated but not heated. Their appearance and odor were then determined and 1 c. c. of the liquor, obtained by crushing the vegetables with the brine and straining the resulting "purée," was used for subcutaneous injection of guinea pigs.*

No growth or toxin formation occurred in string beans either acidified or non-acidified and whether heated or not.

Heavy growth, gas production and toxin formation occurred in peas when unheated; when not acidified and heated 1 hour at 100° C. and when canned in brine containing 4 per cent lemon juice but not heated. No growth or toxin production occurred when they were canned in brine acidified with 4 per cent lemon juice and heated for 1 hour at 100° C.

Corn heavily inoculated with the spores and heated 1½ hours at 100° C., with brine containing 6 per cent lemon juice did not spoil and was not toxic.

Since peas were found to be an excellent medium for growth of *B. botulinus,* another experiment was made for direct comparison of the "one-period-cold-pack" and the "lemon juice" methods of home canning. Canned peas were re-canned, heavily inoculated and treated as follows:

a, no acid and not heated; *b,* brine containing 6 per cent lemon juice but not heated; *c,* same as *a* but placed in water in wash boiler and brought to boiling for 3 hours; *d,* same as *b* but heated at boiling point 1 hour; *e,* same as *d* but quart mason jars used instead of cans. The cans and jars were incubated for 1 year at 37° C.

* The guinea pig inoculations were made by Dr. J. Traum of the University of California, Veterinary Science Division.

All cans in lots *a*, *b*, and *c* spoiled and developed the characteristic *B. botulinus* odor. Lots *d* and *e* did not spoil. Ten cans or jars were used for each test.

This test indicates that the "lemon juice" method is much safer than the "cold-pack-one-period" method of home canning, even when only 1 hour at 100° C. is used in the former and 3 hours at 100° C. in the latter.

We realize that the spores used were not so resistant to heat as some spore cultures later developed by Meyer, Dickson, Esty, Burke, and others. Nevertheless a survival of 3 hours at 100° C. is an evidence of marked resistance to heat.

A spore suspension of *B. botulinus* from Dr. K. F. Meyer was used recently for a series of tests with asparagus and spinach. These spores, according to Dr. Meyer, were able to survive 300 minutes at 100° C., in a medium of pH 7—when at their maximum resistance. Their maximum resistance at pH 7 in glucose broth as determined in our laboratory at the time of our experiments was 275 minutes. However, the incubation period of the heated tubes was not long enough to permit the delayed germination of a few spores that may have survived 275 minutes.

Brines of pH 2.0, 2.4, 2.8, 3.6, 4.5, 5.0, 6.0, and 7.0 were used for the asparagus and brines of pH 2.2, 2.8, 3.4, 4.0, 5.0, and 7.0 for the spinach. One set of brines acidified with citric acid and one with acetic acid was used with each vegetable.

At each pH value four cans were heated at 100° C., for 1 hour, four for 2 hours, and four for 3 hours.

The asparagus developed typical *B. botulinus* spoiling after 1 hour of processing but not after 2 or 3 hours when the pH value after processing had dropped to 4. The same results were obtained with spinach. However, the period of incubation has been less than three months—further incubation may change the results.

In order to produce a pH of 4 or less after processing it was necessary to add to the asparagus at the time of canning a brine acidified with citric or acetic acids to pH 2.8 or less and to the spinach a brine of pH 2.2 or less or to blanch the spinach in acidified water before canning.

The percentage of cans spoiling was irregular. Similar results were observed in the other three experiments with asparagus and spinach.

While the experiments with *B. botulinus* were not so extensive as those with *B. sporogenes*, nevertheless they show that *B. botulinus* is much less resistant to heat in cans of vegetables containing acidified

brines with a final pH value after processing of 4.0 or less than in non-acidified brines under the same conditions. Apparently the critical pH value for *B. botulinus* is lower than for *B. sporogenes* or the thermophile used in these investigations.

SUMMARY AND CONCLUSIONS

1. Brines of relatively low original pH value increased in pH value during exhausting by heat and during processing by heat for moderate lengths of time. This increase was much greater than could be accounted for by diffusion and we are forced to conclude, therefore, that it was caused principally by the action of buffer substances dissolved from the vegetables.

2. The change was greater during exhausting than during subsequent processing.

3. Brines of high initial pH value decreased in pH value, possibly because of formation of weak organic acids such as CO_2, H_2S, etc., and by precipitation of buffer substances.

Brines of low pH value increased in pH value to a maximum during the first part of the heating process; then on further heating decreased in pH value, perhaps for the reasons just given.

4. The increase in pH value of acidified brines in canned vegetables was less in brines acidified with citric and acetic acids than in those acidified with hydrochloric acid, because of difference in buffer effects with these acids.

5. The change in pH during heating was greatest with peas and least with artichokes.

6. The effect of the pH value of the brine on the heat resistance of the spores of *B. sporogenes* and *B. botulinus* and a heat resistant thermophile was very pronounced.

7. The pH value during the heating period, practically equivalent to that found after heating, was found to be more significant in relation to the effect on the heat resistance of spores than was the initial pH value of the brine because the initial pH value changes greatly during heating.

8. Spinach canned with a small amount of acidified brine (ratio of vegetable to brine 7:1) exhibited irregularities with respect to effect of pH value on heat resistance of *B. sporogenes;* probably because the brine failed to reach all parts of the rather tightly packed mass of leaves. However, preliminary blanching of the spinach in acid brine of pH 3 made it possible to easily sterilize it at 100° C., even when heavily inoculated with *B. sporogenes* spores.

9. The results of these investigations cannot be applied directly to commercial canning operations until large scale experiments under factory conditions are made. Nevertheless they show that brines acidified with a small amount of citric or acetic acid greatly reduce the heat resistance of the spores of heat resistant bacteria and that if the decrease in pH value during heating is taken into account, it is possible to sterilize canned vegetables much more easily in acidified brines than in non-acidified brines.

ACKNOWLEDGMENTS

The writers desire to express their appreciation to Professor A. W. Christie for valuable suggestions given during the investigations and to Dr. K. F. Meyer and his associates for aid given in the bacteriological part of the investigations.

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